IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Hiroyuki FUJITA
Serial Number: 09/663,709
Filed: September 18, 2000
For: ANGIOTENSIN CONVERTING ENZYME INHIBITOR

Group Art Unit: 1653
Examiner: Anish Gupta

DECLARATION UNDER 37 CFR 1.132

Honorable Commissioner
Washington, D.C. 20231

Sir,

Hiroyuki FUJITA residing at 13-1, Muroyama 2-chome, Ibaraki-shi, Osaka, JAPAN, declares and states:

1. That he graduated from Department of Applied Biological Science, Faculty of Applied Biological Science, Hiroshima University, Hiroshima, Japan, in the year 1987;

2. That he has been employed in the capacity since 1990 by Nippon Synthetic Chemical Industry Co., Ltd., Central Research Laboratory;

3. That he has been engaged in research and development on Food Science.

4. That he received the degree of Doctor of Agriculture from Kyoto University, Kyoto, Japan in the year 1996;

5. That he is the present inventor, and has read and is familiar with the instant application for United States Letters Patent and the Office Action thereto mailed November 20, 2001.

6. That he conducted the experiments described below in order to demonstrate that without a step of removing "heavy"peptides, the proportion of peptides having a molecular weight of 5,000 is always above 10 % in the compositions obtained from the method of the cited references.

(Materials and Methods)

Samples

Samples 1-8 are prepared as follows.

(Sample 1)

Concentrate was prepared as described in JP 04069398 (page 681 lines 2–6); Five grams of dried bonito was added to 40 ml of water and homogenized sufficiently. The homogenate was boiled at 100°C for 10 minutes, and then the resultant was allowed to stand. To the resultant was added 20 mg of thermolysin to carry out hydrolysis reaction at 37°C and pH 7 for 3 hours. After cooled, the resultant was centrifuged and then concentrated.

(Sample 2)

Supernatant containing peptides was prepared as described in Example 1 of USP 5, 369, 015 (column 3, lines 43-64).

(Sample 3)

Supernatant containing decomposed peptides was prepared as described in Example 2 of USP5, 369, 015 (column 4, lines 55 to 60).

(Sample 4)

Supernatant containing decomposed peptides was prepared as described in Example 3 of USP5, 369, 015 (column 5, lines 18 to 23).

(Sample 5)

Supernatant containing decomposed peptides was prepared as described in Example 4 of USP5, 369, 015 (column 5, lines 49 to 54).

(Sample 6)

Supernatant containing decomposed peptides was prepared as described in Example 5 of USP5, 369, 015 (column 6, lines 25 to 31).

(Sample 7)

Mixture of decomposed peptides was prepared as described in Biosci. Biothch. Biochem., 56 (10) ("Digestion of dried bonito" on page 1541, left column, line 29 to right column, line 5).

(Sample 8)

Mixture of decomposed peptides was prepared as described in Example 1 of JP 06340692 (column 5, lines 12 to 17); Fresh sardine was boiled and then the muscle was obtained. To 500 g of the muscle was added 1 liter of deionized water, and the resultant was homogenized. To the homogenate was added 10 g of pepsin. The resultant was adjusted to pH 2 and incubated at 37°C for 20 hours.

Gel filtration chromatography

Molecular weight distribution of each sample was measured by a gel filtration chromatography. Concretely, using a peptide and a protein [Asp-Gly-Leu-Tyr-Pro (molecular weight 563), neurotensin (molecular weight 1637), ribonuclease (molecular weight 13700) as s standard substance, which have the known molecular weights, a sample was eluted in the following conditions to determine a retention time, and a molecular weight was determined by using a calibration curve obtained by plotting the molecular weights on the ordinate and the

retention times on the abscissa logarithmically. An amount "% by weight" of the components having a molecular weight of at least 5000 is represented as "% area" of the peaks corresponding to components having a molecular weight of at least 5000 after dividing the peaks at the position of the molecular weight 5000 on an elution chromatogram.

(Fraction conditions)

Column : Protein Pak60 (made by Waters) 5 × 250 mm

Mobile phase : 50 % by volume acetonitrile aqueous solution containing 0.1 % by volume TFA (trifluoroacetic acid)

Flow rate : 0.7 mL/min

Detection : RI

Sample amount: 1 mg

(Relation between molecular weight of standard substance and elution time)

Standard substance	Molecular weight	Elution time (minute)
Asp-Gly-Leu-Tyr-Pro	563	31
Neurotensin	1637	26
Ribonuclease	13700	19

In the above-mentioned conditions, since a substance having a molecular weight of 5000 was eluted at 29 minutes, any substance eluted at not more than 29 minutes was regarded as polypeptide components having a molecular weight of at least 5000.

Result and Discussions

As a result of gel filtration chromatography, content of peptides having at least 5000 of molecular weight in samples 1 to 8 are shown in Table 1.

Table 1

Sample No.	Enzyme	Content of peptideshaving at least 5000 of molecular weight (% by weight)
1	Thermolysin	25
2	Thermolysin	21
	Pepsin	26
3	Thermolysin	27
	Pepsin	29
4	Thermolysin	27
	Pepsin	29
5	Thermolysin	30
	Pepsin	30
6	Thermolysin	30
	Pepsin	30
7	Thermolysin	21
	Trypsin	33
	Chymotrypsin	57
	Pepsin	17
	Trypsin + Chymotrypsin	73
	Pepsin → Trypsin	15
	Pepsin → Chymotrypsin	15
	Pepsin → Trypsin + Chymotrypsin	19
8	Pepsin	33

From the result mentioned above, it is clear that the content of peptides having at least 5000 of molecular weight in each of samples 1 to 8 is not at most 10 % by weight.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

This 7th day of February, 2002

by Hiroyuki Fujita

Hiroyuki Fujita

We, the undersigned witnesses, hereby acknowledge that Hiroyuki Fujita is personally known to us and did execute the foregoing Declaration in our presence on:

Date: February 7, 2002 Witness Masaru Takeshita

Date: February 7, 2002 Witness Keiichi Yokoyama